Sulfate reducing capacity of a *qmoABC* deletion in *Desulfovibrio vulgaris* Hildenborough

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DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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Abstract

The sulfate-reducing bacteria (SRB) are a heterologous group of anaerobic bacteria linked by their ability to respire the costly substrate sulfate as an electron acceptor and as a source of sulfur for cellular biosynthesis. All of the SRB organisms, of which *D. vulgaris* is a member, apparently share the same pathway for sulfate reduction, including an activation step involving the conversion of sulfate to adenosine-phosphosulfate (APS), which consumes two ATP equivalents. The enzyme complex involved in the activation step is APS-reductase, comprised of the two proteins, ApsB and ApsA. In *D. vulgaris* the *apsBA* genes are predicted to be the first two genes in a six gene operon. The three genes that immediately follow *apsBA* are *qmoABC* (Quinone-interacting membrane-bound oxidoreductase) that are conserved in all the genomes of SRB sequenced to date. We have deleted these three genes (and a hypothetical protein predicted to be present at the end of the operon, DVU0851) in *D. vulgaris* and monitored the strain's ability to grow in the presence of sulfate or sulfite. Here we describe the method of deleting these four genes and the growth characteristics of the construct. As predicted by its genomic location, the Qmo complex is essential for APS reduction and sulfate respiration but not sulfite respiration.

Background (Figures 1a, 1b)

Two basic means to reduce sulfate: assimilative (used for amino acid synthesis in non-SRB) and dissimilative (used for sulfate respiration in SRB).

D. vulgaris contains the enzymes for both types of sulfate utilization (fig. 1a). The operon containing the genes for dissimilative sulfate reduction, adenylylsulfate reductase, *apsBA*, also contains the genes *qmoABC* (an electron transport carrier) and a hypothetical protein (fig. 1b).

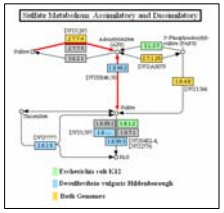

Figure 1a: Sulfate reduction genes in *D. vulgaris* and *E. coli*.

Figure 1b: Operon containing adenylylsulfate reductase genes, *apsBA* and *qmoABC*, in *D. vulgaris*.

Construction of Δ*qmo* strain (Figure 2)

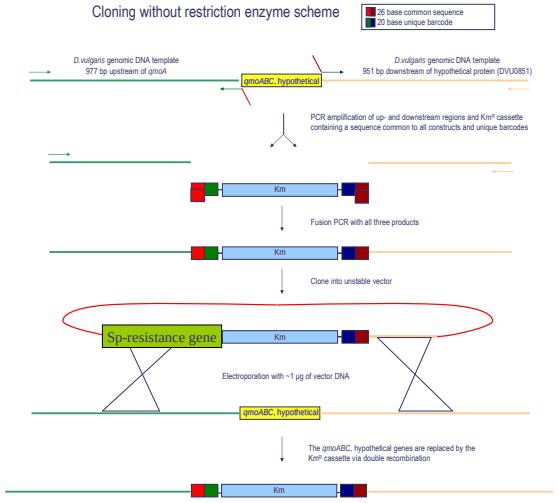

Figure 2: Mutagenesis procedure to obtain Δ*qmo* mutant.

Verification of Δ*qmo* (Figures 3a, 3b)

- Putative Δ*qmo* mutants were selected by resistance to G418 and sensitivity to spectinomycin
- Southern blot verified double-homologous recombination (fig. 3a)
- Verified expression of *apsA* gene with a Northern blot (fig.3b).

* observation of interest – expression of *apsA* in wild-type cells grown in lactate-sulfate appears to be different than the same cells grown in lactate-sulfite (fig. 3b).

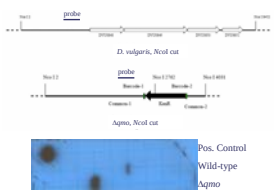

Figure 3a: Theoretical digest and Southern blot of wild-type and a putative Δ*qmo* mutant probed for *apsA*.

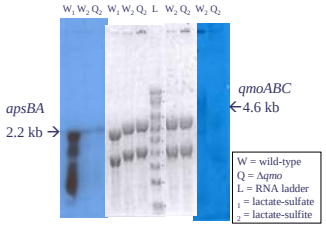

Figure 3b: Northern blots probed with *apsA* or *qmoA*

Growth Characteristics of Δ*qmo* (Figure 4)

- Growth of Δ*qmo* is not possible on lactate-sulfate (fig. 4).
- Growth of Δ*qmo* on lactate-sulfite is reduced (fig. 4) but remains similar to wild-type for lactate-thiosulfate (fig. 4).

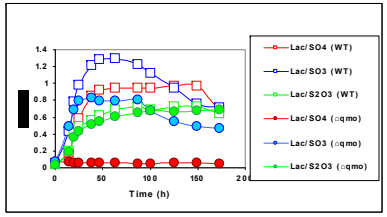

Figure 4: Growth of wild-type (WT) and Δ*qmo* on lactate-SO_4, -SO_3, or -S_2O_3.

Construction of *qmo*⁺ strain (Figure 5)

- In order to verify no additional mutations have contributed to the inability of Δ*qmo* to grow on sulfate, the *qmoABC* and hypothetical genes were re-introduced into the Δ*qmo* strain, making the *qmo*⁺ strain.
- A PCR fragment of the entire operon was captured in a spectinomycin-resistance-containing plasmid and electroporated into Δ*qmo* (fig. 5).

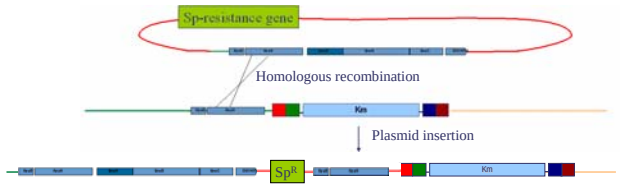

Figure 5: diagram of *qmoABC*, hypothetical protein complementation for Δ*qmo*, yielding *qmo*⁺.

Verification of *qmo*⁺ (Figures 6a, 6b)

- Putative *qmo*⁺ colonies were screened by resistance to spectinomycin and ability to grow on lactate-sulfate medium (fig. 6a).
- Further verification was performed by probing for the *apsA* gene in wild-type, Δqmo, and putative *qmo*⁺ (fig. 6b).

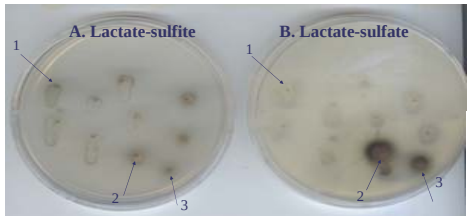

Figure 6a: selection of *qmo*⁺ isolates. A: growth of Δ*qmo* ("1") and two putative *qmo*⁺ isolates ("2" and "3") on lactate-sulfite. B: growth of Δ*qmo* ("1") and two putative *qmo*⁺ isolates ("2" and "3") on lactate-sulfite.

Figure 6b: *apsA*-probed Southern blot of wild-type, Δ*qmo*, and *qmo*⁺.

Growth Characteristics of *qmo*⁺ (Figure 7)

Wild-type *D. vulgaris* and *qmo*⁺ were grown on lactate-sulfate, lactate-sulfite, and lactate-thiosulfate media to compare growth (fig. 7).

- Growth of the *qmo*⁺ is comparable to that of wild-type on lactate-sulfate and lactate-sulfite (fig. 7).

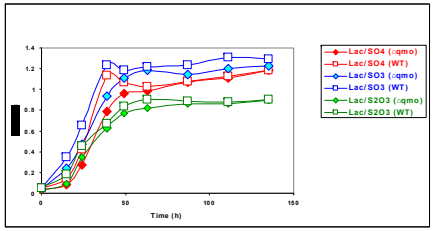

Figure 7: Growth of wild-type (WT) and *qmo*⁺ on lactate-SO_4, -SO_3, or -S_2O_3

Conclusions

- The QmoABC complex is necessary for sulfate reduction in *D. vulgaris*.
- No other transmembrane complex is able to replace the function of the QmoABC complex to deliver electrons to the ApsBA complex.
- The *apsBA* genes are differentially expressed depending on the presence or absence of sulfate.
- Complemetation of qmoABC and hypothetical protein back into the Δ*qmo* strain restores nearly wild-type sulfate reduction capability.

ACKNOWLEDGEMENT

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